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A MORPHOLOGICAL, ALLOZYMIC, AND KARYOTYPIC ASSESSMENT OF THE PHYLOGENY OF SOME LOWER TERMITES (ISOPTERA: KALOTERMITIDAE)

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Abstract.—Ten species of termites in the family Kalotermitidae were examined morphologically, electrophoretically, and chromosomally. The assignment of the recently described species *Neotermes luykxi* Nickle and Collins 1989, to the genus *Neotermes*, originally made solely on morphological grounds, is supported by the electrophoretic data. *Neotermes castaneus*, however, appears to be a sister group to other species of *Neotermes* and *Incisitermes*. Observations on the chromosomes suggest that centric fusions, translocations involving the sex chromosomes, and discrete genome amplification events have all been involved in karyotype evolution in these termites, but a phylogeny based on chromosomal changes alone does not agree with the morphological and electrophoretic data. The observations suggest that chromosomal changes may be too rapid and widespread to be of use in constructing phylogenies in these insects.

Key Words: termites, *Neotermes*, enzymes, chromosomes, cladistics

This morphological, allozymic, and chromosomal survey of several kalotermitid termites from Florida (and a few from elsewhere) was undertaken for two reasons: first, to clarify the systematic position of the newly described species *Neotermes luykxi* (Nickle and Collins 1989); and second, to try to resolve an apparent conflict between the karyotypic relations of some members of the family Kalotermitidae (Luykx and Syren 1979) and the systematic relations as described by Krishna (1961).

Preliminary observations on *Neotermes luykxi* had at first suggested that it might simply be a morphological variant of *Neotermes jouteli* (Banks), but chromosome counts and preliminary electrophoretic data suggested a closer relation to another Florida termite, *Incisitermes snyderi* (Light). A comparison of the new species with a wider range of other kalotermitids was therefore required. In this paper we report the results of an allozymic, morphological and karyotypic comparison of the new species with nine other kalotermitid species.

Early chromosome studies on kalotermitid termites from south Florida (Syren and Luykx 1977, Luykx and Syren 1979) revealed the presence of sex-linked translocations in several species belonging to different genera, but other species in the same genera lacked them. Neither in the presence or absence of these translocations, nor in the chromosome numbers, did there seem to be much correlation between karyotype and systematic position. Therefore this study was undertaken to re-evaluate, using modern cladistic methods, the phylogeny of the

Table 1. Species of termites used in the electrophoretic studies.

Genus, Species	Colony i.d. No.	Collecting Sites	Number of Genomes Sampled
Cryptotermes			
cavifrons Banks	547	Elliott Key, FL	4
Incisitermes			
milleri (Emerson)	357	Mona Island, PR	4
minor (Hagen)	5C	Lafayette, CA	4
schwarzi (Banks)	490, 540	N. Miami, FL	8
snyderi (Light)	541	Hollywood, FL	4
Neotermes			
castaneus (Burmeister)	551	Miami, FL	4
jouteli (Banks)	*	Southern Florida	14
luykxi Nickle & Collins	466, 543	Hollywood, FL	7
mona (Banks)	361A	Mona Island, PR	4
Pterotermes			
occidentis (Walker)	91-N2-125	Tucson, AZ	4

* Colonies 432 (Knight Key), 454 (Bokeelia), 463 (Fakahatchee Strand), 465 (Dania), and 527 (Hollywood).

termites whose karyotypes had already been described in a preliminary way. The karyotypes are described in more detail, and the results are evaluated with regard to the question of the role of chromosome changes in speciation in these insects.

Materials and Methods

Termite colonies were maintained in the laboratory on the wood in which they were collected, for periods of time ranging from one month to two years. For the electrophoretic studies, individual termites were homogenized in one or two drops of dilute buffer (0.03 M Tris-citrate, pH 8.5), and the homogenates were absorbed onto filter paper wicks. Electrophoresis was carried out on 12% horizontal starch gels, using a 1:1 mixture of starch from Electrostarch Co. (Madison, Wisconsin) and Connaught Laboratories Ltd. (Willowdale, Ontario, Canada), with either the Tris-citrate buffer system (pH 8.5) described by Ridgway et al. (1970), or 0.01 M Tris-citrate at pH 6.7. Gel slices were stained according to standard procedures described by Yang (1971) and Harris and Hopkinson (1976). In all cases where there was any doubt about the relative migration rates of electromorphs from different species, the samples from the questionable species were re-run side-by-side.

An average of 5 worker termites from each colony were used. Since each colony is a single family (the king, the queen, and their offspring; Santos and Luykx 1985), a single worker from a colony represents two parental genomes, a sample of two workers represents (on average) three parental genomes, and in a sample of 5 workers the probability is .93 that all four parental genomes are represented. The number of genomes sampled for each species, along with the locations of collecting sites, is given in Table 1.

Chromosome preparations of meiotic and mitotic cells were made from the testes of reproductive males by methods described earlier (Luykx and Syren 1979, Luykx 1983). Meiotic cells provided the best material for determining the presence or absence of translocations involving sex chromosomes, while mitotic cells were best for determining the number of acrocentric and metacentric chromosomes. For a few species, only a small amount of material was available, and occasionally the quality of the chromosome

preparations from these species left some uncertainty as to the exact number of metacentrics present in the karyotype. The numbers given represent our best estimates of metacentrics vs. acrocentrics; we consider it unlikely that they are in error by more than ±1 (haploid), a margin of error that does not affect our general conclusions.

For the purposes of this study, the chromosomal data reported here have been combined with previously published observations on the chromosomes of *Cryptotermes cavifrons, Incisitermes milleri, I. schwarzi, I. snyderi, Neotermes castaneus,* and *N. jouteli* (Luykx and Syren 1979). The observations on the chromosomes of *Incisitermes minor, Neotermes luykxi, N. mona,* and *Pterotermes occidentis* are new. The observations on the chromosomes of *Mastotermes darwiniensis* Froggatt confirm these previously published by Bedo (1987).

Morphological characters were determined from preserved specimens in the collection of the U.S. National Museum of Natural History, Washington, D.C.

The morphological and allozymic data sets were analyzed, both separately and combined, with the PAUP (Phylogenetic Analysis Using Parsimony) package (versions 2.4 and Beta test 3.0) written by David L. Swofford (Illinois Natural History Survey, 607 East Peabody Drive, Champaign, Illinois 61820). The morphological data were coded and analyzed as an ordered data set, with the transformation series based on outgroup comparison. The allozyme data were analyzed as an unordered data set. To ensure equal clustering power among characters with disparate numbers of states, the characters were weighted according to the number of states. Because each state has potential clustering power, a multi-state character has inherently more clustering power than a simple two-state character (Cranston and Humphries 1988). Therefore multi-state characters were down-weighted to be equal to two-state characters. Best estimates of relationship were obtained using both BRANCH SWAPPING (GLOBAL) and BRANCH AND BOUND subroutines for comparison.

Mastotermes darwiniensis was employed as the outgroup for the morphological analysis and the combined data analysis. No allozyme data were available for *M. darwiniensis,* so no outgroup was employed in the allozyme analysis. Instead, the allozyme tree was rooted at the midpoint and interpreted as an unrooted tree or network. Because a midpoint root is simply placed halfway between the two farthest points on a tree it does not affect character-state transformation series, and its removal results in a network.

Results

Morphology.—Thirteen morphological characters were examined, seven from imagoes and six from soldiers. When these characters were used for phylogenetic analysis, using *Mastotermes darwiniensis* as the outgroup, a single most-parsimonious cladogram was obtained (Fig. 1A). With the exception of *Neotermes castaneus,* the results are reasonably consistent with the taxonomy as reflected in the generic divisions. Examination of the morphological data matrix (Table 2) reveals that morphological characters are useful in indicating phylogenetic relations at the generic level, but (especially in the genera *Neotermes* and *Incisitermes*) are not generally useful in establishing phylogenies at the species level, nor even in distinguishing species within genera.

For most of the morphological characters examined, *Neotermes* species appear to be more similar than *Incisitermes* species to *Mastotermes darwiniensis* (family Mastotermitidae), the species used as the outgroup for the cladistic analysis. *M. darwiniensis,* because of several cockroach-like characteristics, is generally considered to be the species most similar to the original ancestors of modern termites (McKittrick 1965, Grassé 1986).

The morphology of *Neotermes castaneus*

Table 2. Morphological traits of some kalotermitid termites compared with *Mastotermes darwiniensis*. *Neotermes* species names: cas, *castaneus*; jou, *jouteli*; luy, *luykxi*; mon, *mona*. *Incisitermes* species names: mil, *milleri*; min, *minor*; sch, *schwarzi*; sny, *snyderi*; Pt. occ, *Pterotermes occidentis*; C. cav, *Cryptotermes cavifrons*; M. dar, *Mastotermes darwiniensis*.

Imago characters: 1: Left mandible, anterior margin of second marginal tooth (1) equal to or (2) longer than posterior margin of first marginal tooth; 2: Right mandible, posterior margin of second marginal tooth (1) equal to or (2) longer than molar plate; 3: Wing, median vein (1) weakly or (2) strongly sclerotized; 4: Wing, median vein (1) closer to radial sector than to cubitus, or (2) midway between radial sector and cubitus; 5: Wing, median vein (1) extends to tip unbranched, (2) or branched, or (3) joins radial sector at two-thirds length of wing; 6: Wing, veins (1) latticed apically or (2) not; 7: Foot (1) with or (2) without arolium.

Soldier characters: 8: Pronotum, shape of anterior margin, (1) concave or (2) incised; 9: Pronotum anterior margin (1) smooth or (2) serrated; 10: Pronotum, posterior margin (1) concave or (2) truncate; 11: Antennal segment no. 3, (1) similar to fourth, (2) greater than fourth + fifth, or (3) greater than fourth + fifth + sixth; 12: Eye pigment (1) absent, (2) slight, or (3) heavy; 13: Head shape (1) oval or pyriform, (2) elongate or reticulate, or (3) phragmotic.

Character Number	*Neotermes*				*Incisitermes*				*Pt. occ*	*C. cav*	*M. dar*
	cas	*jou*	*luy*	*mon*	*mil*	*min*	*sch*	*sny*			
Imagoes											
1. Left mand.	1	1	1	1	2	2	2	2	1	2	1
2. Right mand.	1	1	1	1	2	2	2	2	1	2	1
3. Wing vein sc.	1	1	1	1	2	2	2	2	2	1	1
4. Wing vein pos.	1	1	1	1	2	2	2	2	2	2	1
5. Wing m. vein	2	1	1	1	2	2	2	2	1	3	1
6. Wing v. latt.	1	1	1	1	2	2	2	2	2	2	1
7. Foot	1	1	1	1	1	2	1	1	2	1	1
Soldiers											
8. Pron. s. ant.	1	1	1	1	2	2	2	2	2	2	1
9. Pron. ant. m.	1	1	1	1	2	2	2	2	1	1	1
10. Pron. post.	1	2	2	2	2	2	2	2	1	1	1
11. Ant. segm.	1	2	2	2	2	3	3	2	2	1	1
12. Eye pigm.	1	3	3	1	1	2	2	2	1	1	1
13. Head shape	2	2	2	2	2	2	2	2	1	3	1

is generally similar to that of other species in the genus, but it differs from other *Neotermes* in three of the thirteen traits summarized in Table 2: in imago wing venation, in the relative length of the third antennal segment, and in the shape of the pronotum in soldiers (characters 5, 10, and 11). According to the results of the cladistic analysis (Fig. 1A), the last two of these traits are primitive traits retained from an ancestor (i.e. like the traits in the outgroup species), while the wing venation (character 5) is a derived trait similar to that in the genus *Incisitermes*. The cladogram suggests that the similarity of this trait in *Incisitermes* species and *Neotermes castaneus* is a result of convergent evolution.

Neotermes luykxi differs morphologically from *N. jouteli* only slightly. It is slightly smaller in size and has a somewhat narrower soldier postmentum (Nickle and Collins 1989). As described below, however, the two species can be reliably distinguished on the basis of allozyme patterns and chromosome number.

Allozymes.—The electrophoretic data are summarized in Table 3. Phylogenetic analysis of the allozyme data alone resulted in 7 equally parsimonious trees. These 7 trees could be combined via majority-rule con-

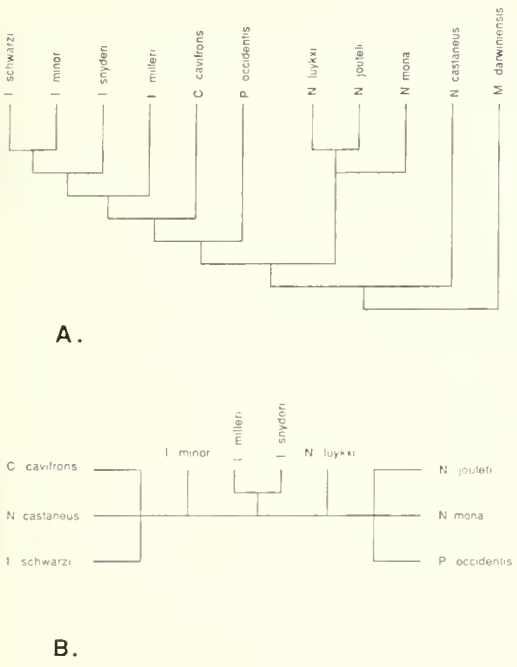

Figure 1

Fig. 1. Hypotheses of relationships based on independent analyses of the morphological and biochemical data. A. Cladogram based on the morphological data. It is the single most parsimonious hypotheses, with a consistency index (C.I.; Kluge and Farris 1969) of .708. B. Network based on allozyme data. The trichotomies are the result of seven most parsimonious trees (C.I. = .879) combined by the majority rule consensus method.

sensus to yield a single general hypothesis (Fig. 1B). It is clear that the morphological and allozyme results are not congruent (compare Fig. 1A with Fig. 1B). The morphology supports the monophyly of *Incisitermes*, the paraphyly of *Neotermes*, and the sister status of *N. jouteli* and *N. luykxi*. But the unrooted allozyme tree suggests that neither *Incisitermes* nor *Neotermes* are monophyletic, and does not support a *N. jouteli-luykxi* sister group.

However, combining data from several studies into a single matrix is often preferable to consensus methods for reconstructing phylogenies (Miyamoto 1985, Hillis 1987). Accordingly, the allozymic and morphological data were combined in a single matrix, and this resulted in a single most parsimonious cladogram (Fig. 2). The combined result is entirely congruent with the morphological result. In the combined cladogram all the nodes are well defined and all the terminal taxa are well delineated.

Of the 30 characters, 12 (1, 2, 4, 6, 8, 9, 14, 16, 17, 18, 24, and 25) were perfect fits (C.I. = 1.0; see legend to Fig. 1) to the final hypothesis (Fig. 2). Of these 12, only one character (24) had no synapomorphic content; the other 11 were all informative. The morphological characters were not good delineators at the species level: only 3 of the 10 taxa—*Cryptotermes cavifrons*, *Pterotermes occidentis*, and *Neotermes castaneus*—were delineated by any morphological characters, and even for these 3 taxa over half of the autapomorphies were allozyme characters. The rest of the taxa were identified as unique only by allozyme characters. (Every HTU (hypothetical taxonomic unit) node is, however, defined by at least one morphological character.)

The combined tree is in general agreement with the one presented by Krishna (1961, fig. 81) for the genera of the family Kalotermitidae, except for the position of *Pterotermes*, which is here joined with *Incisitermes* and *Cryptotermes* to form a monophyletic group, whereas Krishna put *Pterotermes* on a branch together with *Neotermes*.

The new species *Neotermes luykxi* could be distinguished from the morphologically similar *N. jouteli* at nine of the seventeen allozymic loci examined. This finding, along with the clear chromosome differences described below, leaves no doubt that the two are distinct species. Nevertheless, *N. luykxi* did appear most similar to *N. jouteli* among the other species examined by electrophoresis, being indistinguishable from it at eight of the seventeen loci studied. Three of these loci (characters 15–17: AC-C, ADH, and ALD) are synapomorphies for *N. luykxi* and *N. jouteli*, an additional two (characters 25

Table 3. Allozyme (enzyme) data obtained by starch gel electrophoresis on ten species of kalotermitid termites. For each locus, lower numbers indicate forms of the enzyme that migrated more slowly on the gels, higher numbers forms that migrated more rapidly. All enzymes were anodal, except for those designated "-C," which appeared on the cathodal side of the origin. 0 = enzyme not detected; X = not tested.

Species abbreviations as in Table 2.

AC, aconitase; ADH, alcohol dehydrogenase; ALD, aldolase; GAM, galactosaminidase; GK, glucokinase; GNDH, gluconate dehydrogenase; GPI, glucose phosphate isomerase; GR, glutathione reductase; LAP, leucine aminopeptidase; MDH, malate dehydrogenase; ME, malic enzyme; PEP, glycyl-leucine peptidase; PGM, phosphoglucomutase; SOD, superoxide dismutase; XDH, xanthine dehydrogenase.

Character Number	Locus	*Neotermes*				*Incisitermes*					
		cas	*jou*	*luy*	*mon*	*mil*	*min*	*sch*	*sny*	*Pt. occ*	*C. cav*
14.	AC	1	3	5	1	2	2	4	6	2	2
15.	AC-C	4	2	2	4	X	3	3	2	1	3
16.	ADH	0	2	2	1	X	0	4	5	3	6
17.	ALD	3	5	5	2	X	6	3	3	4	1
18.	GAM	1	1	1	1	2	3	4	3	0	3
19.	GK	6	1	3	3	4	5	6	3	4	2
20.	GNDH	2	4	1	5	6	1	7	6	3	2
21.	GPI	6	3	4	4	3	6	5	4	1	2
22.	GPI-C	2	2	1	2	1	3	4	1	2	1
23.	GR	4	3	2	1	2	2	2	0	3	3
24.	LAP	4	1	1	3	X	1	1	1	0	2
25.	MDH	1	6	6	6	4	7	4	2	5	3
26.	ME	2	2	2	1	5	6	3	4	5	3
27.	PEP	2	6	5	6	5	7	3	1	4	4
28.	PGM	6	4	5	4	5	3	5	5	1	2
29.	SOD	2	4	4	4	5	1	2	3	4	3
30.	XDH	1	2	3	1	4	3	2	3	0	3

and 29: MDH and SOD) are synapomorphic for the group that includes *N. mona*, and the remaining three (characters 18, 24, and 26: GAM, LAP, and ME) appear to be primitive characters shared by other species as well.

Chromosomes.—*Neotermes luykxi* and *N. jouteli* can be readily distinguished on the basis of their chromosomes (Figs. 3e, f). Not only are the chromosome numbers different (2n = 45 and 56, respectively), but the N.F. ("nombre fondamental," the number of major chromosome arms) is different also (haploid, 25 and 28, respectively). Furthermore, *N. luykxi* populations have a sex-trivalent; no sex-multivalents of any kind have been seen in *N. jouteli*.

The results of the chromosome study for all species are summarized in Table 4; several examples are illustrated in Fig. 3. The karyotypes of the ten kalotermitid species examined for this study show considerable variation. Diploid chromosome numbers range from 28 (*Incisitermes milleri*) to 79 (*Pterotermes occidentis*). The diploid chromosome sets of some species are composed entirely of acrocentrics, while others contain, in addition to acrocentrics, from 3 to 22 metacentrics. Some species are without morphologically differentiated sex chromosomes, while others have multiple sex chromosomes that in male meiosis form chains or rings containing from 3 to 14 chromosomes.

The total number of major chromosome *arms* (the "nombre fondamentale," N.F.) in different species shows relatively little variation compared to the chromosome number itself (see Table 4). For example, the haploid chromosome numbers of *Incisitermes schwarzi, N. mona*, and *N. jouteli* are 16, 23 and 28, respectively, while the total num-

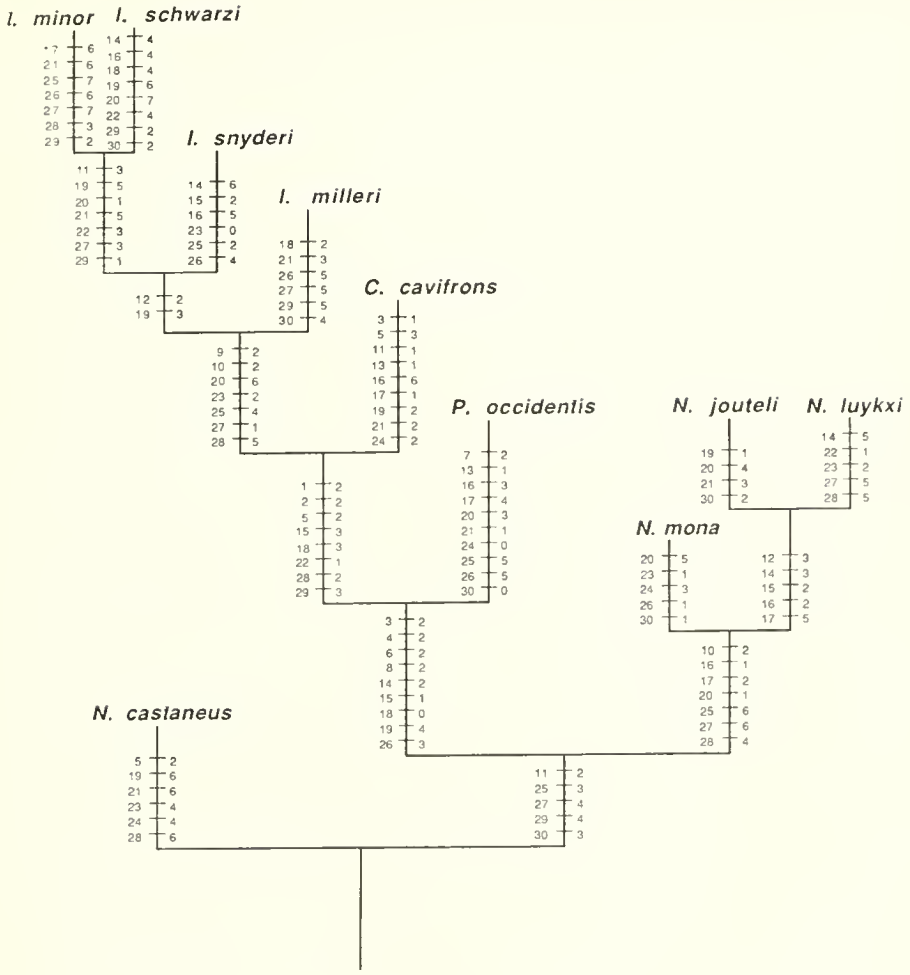

Figure 2

Fig. 2. The final cladogram constructed from the combined morphologic and allozymic matrix. Excluding uninformative characters, the C.I. is .780. The steps associated with the branch connecting the outgroup to the most recent common ancestor of the study group are not shown because they are unimportant in defining the relationships of the study group. Numbers to the left of the hash marks represent characters; numbers to the right of the hash marks represent character states.

bers of major chromosome arms for these species are 27, 27, and 28.

When the total numbers of major chromosome arms are compared among the different species, the numbers appear to fall into distinct groups. *I. milleri* has N.F. = 14; most of the other *Incisitermes* and *Neotermes* species have an N.F. ranging from 25 to 28; and *Pterotermes occidentis* and *Mastotermes darwiniensis* have N.F. = 49 and 52, respectively.

It is possible to order the chromosome changes in plausible evolutionary sequences, assuming for example that the N.F. changes from low to high by some sort of amplification process, that centric fusions increase the number of metacentric chromosomes at the expense of acrocentric chro-

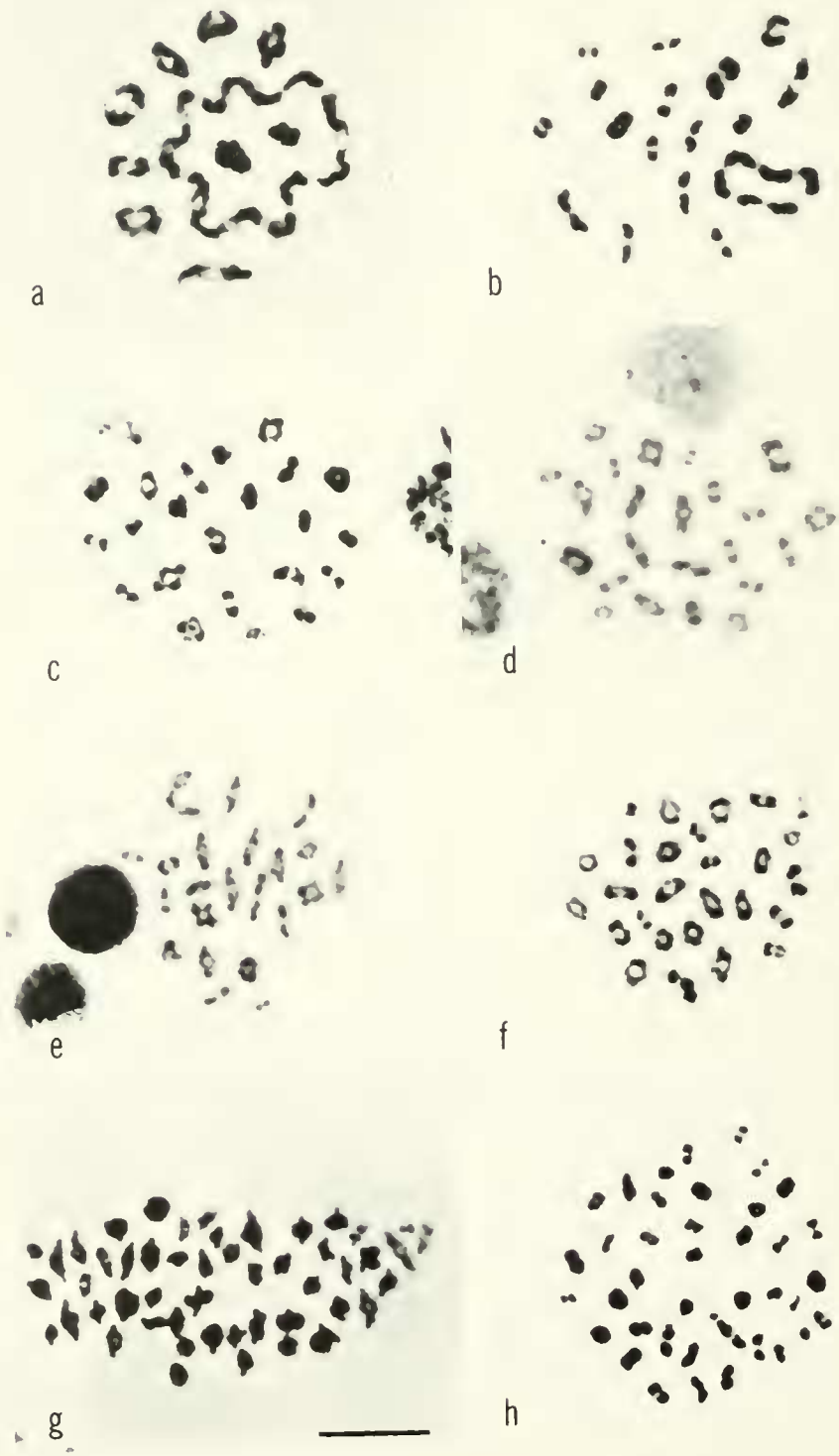
a
b
c
d
e
f
g
h

Table 4. Chromosomes of several species of kalothermitid termites and of *Mastotermes darwiniensis*. The columns in the table are: *a*, species name; *b*, the reference for the karyotype (1, this report. 2, Luykx and Syren 1979; 3, Bedo 1987); *c*, diploid chromosome number in males; *d*, approximate number of metacentric (two-armed) chromosomes in the diploid set; *e*, the N.F. ("nombre fondamental," or total number of chromosome arms) of the haploid set; *f*, the number of recognizable sex chromosomes (usually multivalent).

a Species	*b* Ref.	*c* 2n	*d* Meta	*e* N.F.	*f* Sex Chrom.
Neotermes castaneus	2	38	14	26	VI
Neotermes jouteli	2	56	0	28	—
Neotermes luykxi	1	45	3	25	III
Neotermes mona	1	46	8	27	—
Incisitermes milleri	2	28	0	14	—
Incisitermes minor	1	37	11	25	III
Incisitermes schwarzi	2	32	22	27	XIV
Incisitermes snyderi	1, 2	45	8	27	I
Pterotermes occidentis	1	79	17	49	III
Cryptotermes cavifrons	2	40	2	21	—
Mastotermes darwiniensis	1, 3	98	6	52	—

mosomes, and that a simple sex-chromosome pair is built up to multivalent rings and chains by means of successive centric fusions and translocations between sex chromosomes and autosomes. On such assumptions plausible phylogenies based on chromosomal changes alone can be constructed; an example is illustrated in Fig. 4.

It is clear that this phylogeny bears little relation to that based on morphology and allozymes (compare Fig. 4 with Figs. 1 and 2). As explained in the Discussion, there is reason to think that *chromosomal changes* may occur more frequently and become established in populations more rapidly than the *genic changes* that accompany speciation and that are reflected in the morphological and allozymic variation between species. Therefore the chromosomal data were not added to the allozymic and morphological data matrix, and no attempt was made to arrive at a consensus phylogeny using the chromosomal data.

Discussion

One of the reasons for undertaking this study was to clarify the systematic relationship of a new termite discovered in south Florida in 1984. Initially thought to be simply a size variant of *Neotermes jouteli*, it became the subject of a careful morphometric study (Nickle and Collins 1989) when it was later found to have a chromosome number different from that of *N. jouteli*. On the basis of the morphometric study (Nickle and Collins 1989), it was recognized as a distinct species and named *Neotermes luykxi*. The present study established clearly that, on the basis of both chromosome number and enzyme differences, it is a species distinct from *N. jouteli*.

A difference in chromosome number alone is not sufficient to establish species status. "Chromosomal races" of the same species may also have different chromosome numbers as a result of variation in the number of centric fusions (John 1983). But

←

Fig. 3. Male meiosis in several lower termites. Magnification is approximately the same for all cells, about 1400×. Bar = 10 μm. a, *Incisitermes schwarzi*, 9 bivalents and a sex-multiple of 14 chromosomes; b, *Neotermes castaneus*, 16 bivalents and a sex-multiple of 6 chromosomes; c, *Incisitermes snyderi*, 22 bivalents and a sex-univalent, upper left; d, *Neotermes mona*, 23 bivalents; e, *Neotermes luykxi*, 21 bivalents and a C-shaped sex-trivalent, at top; f, *Neotermes jouteli*, 28 bivalents; g, *Mastotermes darwiniensis*, 49 bivalents; h, *Pterotermes occidentis*, 38 bivalents and a linear sex-trivalent, just below and to the right of center.

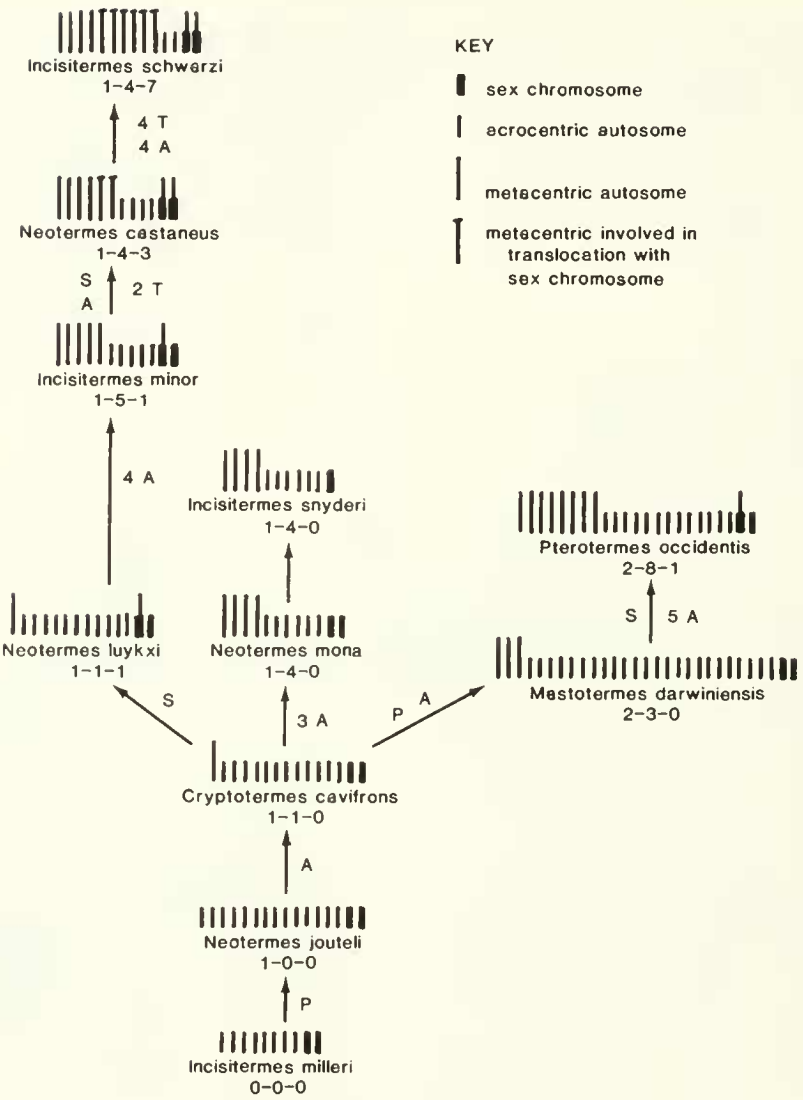

Fig. 4. Schematic representation of ordered karyotype changes in 11 species of lower termites (10 Kalotermitidae, 1 Mastotermitidae), based on the data in Table 4. Arrows are labeled by the following karyotypic changes: P, polyploidization; A, autosomal centric fusion; S, sex-chromosome-autosome centric fusion; T, translocation between autosome and sex-chromosome. The 3-digit formula under each species name indicates for each karyotype (ploidy level)–(haploid number of autosomal metacentrics)–(haploid number of sex-chromosome metacentrics). This tree was constructed by hand, minimizing genome amplification events and avoiding centric fissions on the assumption that these are much less frequent than centric fusions and translocations in karyotype evolution. This and other parsimonious trees of karyotype evolution (constructed by PAUP) are not congruent with the trees (Figs. 1 and 2) derived from morphological and allozyme characters (see text).

in such cases the N.F., the number of major chromosome arms, is the same for all races. This is not the case here; the haploid N.F. for *luykxi* is 25, while that for *jouteli* is 28. This indicates that the two types are not simply different populations of a single polytypic species related by centric fusions. Moreover, fusions between autosomes and sex chromosomes have occurred in *luykxi* but are so far unknown in *jouteli*.

These chromosomal differences, along with the enzyme differences described in this

paper (9 out of 17 loci studied) and the slight but definite morphological differences described by Nickle and Collins (1989), leave little doubt that *luykxi* and *jouteli* do not share a common gene pool, and are therefore different species. The phylogenetic relationships of the two species, as reflected in the cladogram based on the combined morphological and electrophoretic data (Fig. 2), indicate that the two species are nevertheless closely related, and therefore *luykxi* is appropriately included in the genus *Neotermes*.

The cladogram (Fig. 2) is based on 13 morphological characters and 17 enzyme characters. It might be argued that the relatively small number of individuals sampled in the electrophoretic studies could bias the cladogram, if numerous enzyme polymorphisms went undetected, and apparently fixed differences between species were in fact simply different allelic forms uncovered as a result of chance sampling among a small number of genomes.

For several reasons, it is unlikely that many of the species differences represent simply allelic differences in polymorphic populations, and that the phylogenetic tree topology is thereby significantly biased. First, levels of enzyme polymorphism in the lower termites are probably low. In the only kalotermitid species studied extensively to date, *Incisitermes schwarzi*, Santos and Luykx (1985) found only 4 polymorphic loci out of 23 studied (17%). Limited though the samples of each species in the present study were, there were no other clear cases of intraspecies polymorphism, indicating that levels of polymorphism in the other species are generally low also.

Secondly, when 4 genomes are sampled for each species (the minimum number sampled for each species in this study), the probability that two species will appear to have fixed differences when in fact they both are polymorphic at a given locus, is not very high. It can be calculated, for example, that if two species are both polymorphic at a locus, and alternate alleles each occur at a frequency of .95 in the two different species, then the probability is .66 that, when 4 genomes are sampled from each species, one species will appear to be fixed for one allele and the other species will appear to be fixed for the other allele. This means that under these special circumstances—approaching a state of fixed species differences—about ⅔ of the truly polymorphic loci will show up as fixed differences in the two species; that is, only about 2 loci out of the 17 loci studied, assuming that *Incisitermes schwarzi* is fairly representative of the family (see above). If the two alleles are both equally frequent at a given locus in each of two species, the probability is less than 1% that the two species will appear to be fixed for the different alleles.

Finally, it has been shown that when a relatively large number of loci are used, and the fraction of loci that are polymorphic is relatively low (as appears to be the case here), samples as small as even single individuals from each species give cladogram topologies that are not any different from those obtained when larger species samples are used (Hillis 1987; see also Gorman and Renzi 1979).

Admittedly, larger sample sizes for each of these species would resolve these questions. But the above considerations make it improbable that failure to detect enzyme polymorphisms because of limited sample sizes significantly affected the topology of the cladogram.

The cladogram presented in Fig. 2, combining both morphological and electrophoretic data, was rooted using *Mastotermes darwiniensis* as the outgroup. Samples of this species were unfortunately not available at the time of the electrophoretic studies, so the rooting of the tree is based on the morphological data alone. In view of the general agreement (similar consistency indices) between the morphological and electrophoretic data, however, it seems unlikely that the addition of electrophoretic data from *Mastotermes* would significantly alter the topology of the tree. This is a point that

can be investigated more thoroughly in future studies.

The phylogeny as presented in Fig. 2 is in general agreement with that proposed by Krishna (1961) on the basis of classical morphological studies. There are, however, two differences. The first is that *Pterotermes occidentis* is phylogenetically related to the *Incisitermes-Cryptotermes* branch of the kalotermitids, not to the *Neotermes* branch as Krishna supposed. The association of *P. occidentis* with *Incisitermes* and *Cryptotermes* rather than *Neotermes* is supported by 9 derived characters, 7 of which are shared with at least one other species of *Incisitermes*, and 3 of which are shared with all the *Incisitermes* species studied. These numbers make it highly improbable that *Pterotermes* is more closely related to *Neotermes* than to *Incisitermes* (see Felsenstein 1985).

The second difference with Krishna's phylogeny is that *Neotermes* appears to be paraphyletic. *Neotermes castaneus* (unfortunately the type species for the genus) in fact appears in Fig. 2 as a sister group to the other *Neotermes* species and to *Incisitermes* species. The data indicate that some revision of the taxonomy of this branch of the Kalotermitidae is required, but it seems premature to revise it until more extensive studies, including more members of the genus, are carried out.

It is interesting that the morphological characters and allozymic characters appear to define phylogenetic groupings at different levels: species are defined more by their unique allozyme characters than by their morphology, while the morphological characters tend to define higher taxa (genera). This tendency has also been observed in other groups of animals (e.g. see fig. 4 in Hillis 1987). The observation suggests that allozymic changes frequently accompany speciation, while morphology is more conserved—a tendency that might be expected if single base changes are responsible for allozyme differences, while significant morphological differences require more extensive genetic repatterning.

Some investigators (e.g. Miyamoto 1983) have treated the karyotype as a single character with multiple states (arising from centric fusions, pericentric inversions, etc.), and have combined karylogical data with the morphological and electrophoretic data to generate phylogenetic trees. Treating chromosomal data in this way, however, assumes that chromosomal changes occur at approximately the same rates and play approximately the same role in speciation as do the gene mutations that lead to changes in morphology and allozymes. Rates of structural changes in chromosomes, however, are several orders of magnitude higher than rates of gene mutations (Jacobs 1981, Van Dyke et al. 1983, Hook et al. 1984). The role of chromosomal changes in speciation is still a controversial subject (e.g. Sites and Moritz 1987), and it therefore seems better to treat chromosomal changes separately, and not combine them with morphological and allozyme data.

It is theoretically possible to construct a separate phylogeny based on the chromosomes of the ten kalotermitid species studied here and of *Mastotermes darwiniensis* (Fig. 3). As outlined in the following paragraphs, the changes that have apparently led to the karyotypic differences between these species are (i) an increase in number of major chromosome arms, possibly by a process akin to polyploidization; (ii) centric fusion between autosomal acrocentrics; (iii) centric fusion between sex chromosomes and autosomes; and (iv) whole-arm translocation between autosomal and sex-chromosomal metacentrics.

Evolutionary polyploidization in animals is very rare (White 1973), and it is uncertain whether this process has really occurred in these termites. But the numbers of major chromosome arms (N.F.) in the species studied here seem to fall into distinct categories: *Incisitermes milleri* has N.F. = 14, most of the other *Incisitermes* and *Neotermes* species have N.F. ranging from 25 to 28, and *Pterotermes occidentis* and *Mastotermes darwiniensis* have N.F. = 49 and

52, respectively. This is almost a doubling series and suggests, if not polyploidization, at least distinct evolutionary episodes of amplification.

The karyotypes of many of these termite species consist of mixtures of acrocentric and metacentric chromosomes. As would be expected if centric fusions or fissions were important in the karyotypic changes exhibited by these termites, metacentric chromosomes are in general about twice the size of acrocentric chromosomes. And even if the absolute chromosome numbers differ, species with similar N.F. have similar DNA contents. The haploid DNA content of *Neotermes jouteli* is 1.30 pg, almost identical with that of *Incisitermes schwarzi*, 1.35 pg (Luykx, unpublished data). While the haploid chromosomes numbers of these two species are quite different, *N. jouteli* with n = 28 (all acrocentrics) and *I. schwarzi* with n = 16 (5 acrocentrics and 11 metacentrics), the N.F. for these two species is almost the same (28 and 27, respectively). Differences in chromosome number are probably due primarily to centric fusions rather than centric fissions, since fusions are much more common than fissions among orthopteroid insects in general (Hewitt 1979), and because there is little doubt that fusions are responsible for the origin of the sex-trivalents in *Neotermes luykxi, Incisitermes minor,* and *Pterotermes occidentis,* as well as in other kalotermitid species (Luykx and Syren 1979).

The formation of the multivalent rings seen in male meiosis in *Neotermes castaneus* and *Incisitermes schwarzi* can be accounted for by a series of whole-arm translocations between autosomal and sex-chromosomal metacentrics. Starting with a metacentric pair of sex chromosomes (which may themselves have arisen by centric fusions), each successive translocation of a sex chromosome with an autosome would increase the size of the sex-multivalent by two chromosomes (Syren and Luykx 1981). Thus, one translocation would give a ring of 4 chromosomes, an additional translocation would give a ring of 6, and so on. Evidently, two such translocations have occurred in *N. castaneus*, and a total of 6 such translocations have occurred in the *I. schwarzi* population used in these studies (see Luykx and Syren 1979 and Luykx 1987 for other translocation variants in this species).

It seems likely that a karyotype consisting entirely of acrocentric chromosomes was the ancestral condition. This karyotype is the most common one among the kalotermitids (Luykx and Syren 1979, Luykx 1990). The chromosomal variations seen in these termites, then, in accord with the considerations discussed above, can be understood as arising from a limited number of processes acting on a primitive all-acrocentric karyotype: the amplification of the number of chromosome arms (perhaps by polyploidization-like events), the fusion of centromeres (between autosomes and sex chromosomes as well as among autosomes), and the translocation of whole arms between autosomal and sex-chromosomal metacentrics.

A phylogeny of chromosome changes, based on the data in Table 4, can be constructed on the above principles. A simple phylogeny is shown in Fig. 3. It is obvious that this and other parsimonious trees of karyotype evolution (constructed by PAUP) bear little relation to the cladogram derived from morphological and allozyme characters (Fig. 2). The most reasonable explanation for the discrepancy is that chromosome arrangements in these insects are too labile to be good indicators of phylogeny. In other words, chromosome changes may arise and be fixed in populations more rapidly than the speciation events themselves, a view supported by the extensive karyotype variation also observed *within* these species (Syren and Luykx 1981, Luykx 1983, Luykx 1987).

Similar karyotype modifications may therefore occur independently on separate branches of the "true" phylogeny (here assumed to be approximated by Fig. 2). Thus,

since there is no obvious mechanism for halving genome size in a single step, discrete genome amplification events (polyploidization?) would have to have occurred separately on all branches except the one leading to *Incisitermes milleri*. Autosomal centric fusions appear to have occurred to varying extents on different branches, giving the same number of autosomal metacentrics in *Incisitermes snyderi* and *Neotermes mona*, for example, quite independently. Similarly, frequent centric fusions and repeated translocations between metacentric sex chromosomes and metacentric autosomes, to give multivalent rings in male meiosis, appear to have occurred independently on separate branches leading to *Neotermes castaneus* and *Incisitermes schwarzi*.

It seems likely that these processes of karyotype modification—approximate doubling of chromosomal material, centric fusion, and whole-arm translocations—are all widespread and common enough that virtually every lineage is subject to them. The different karyotypes that are currently observed in these various species are therefore probably simply the outcome of variations in the frequency with which these chromosome changes occur within lineages, and in population factors that affect the likelihood that the changes will be fixed.

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